

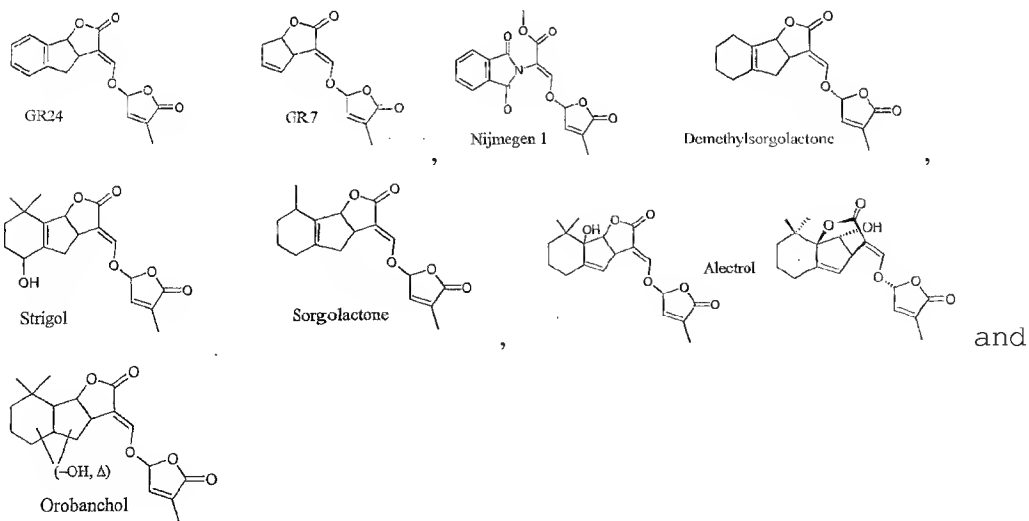
REMARKS

Claims 23-45 remain in this application. Claims 31-44 are withdrawn. Claim 23 is currently amended and new claim 45 has been added. Support for the amendments can be found in the specification and original claims as filed. No new matter has been added.

**CLAIM REJECTIONS - 35 USC § 112**

At page 2, the Office Action rejects claims 23-30 under 35 U.S.C. §112, first paragraph, enablement requirement. Applicants respectfully traverse the rejection.

Claim 23 is directed to a method of treating arbuscular mycorrhiza (AM) fungi that includes contacting the fungi with an agent that stimulates the development and/or growth of the AM fungi. The stimulating agents are the following nine strigolactones:



The Office Action contends that the specification fully enables such a method utilizing GR24 and/or GR7 but not for the other compounds Nijmegen 1, Demethylsorgolactone, Strigol, Sorgolactone, Alectrol and Orobanchol. The Office Action cites several of the Wands factors in reaching its decision. Applicants respectfully disagree with this position.

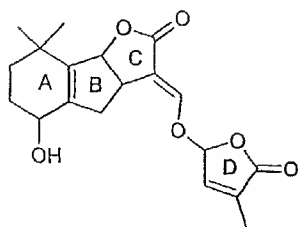
Breadth of the claims

The Office Action states that the claims are very broad in the sense that they encompass "many stimulating agents." Claim 23, however, recites only nine specific stimulating agents. Moreover, these agents are closely related members of the strigolactone family having a defined structure.

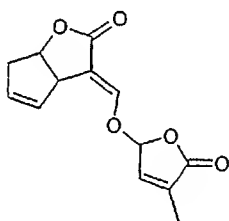
As detailed in the specification, GR7 and GR24 are synthetic analogs of natural compounds belonging to the strigolactone family. This family includes, for example, the natural compounds strigol, alectrol, sorgolactone, and orobanchol.

Recently, the synthesis of GR7 and GR24 can make these compounds easier to obtain in larger amounts than the natural compounds but the effects found with GR7 and GR24 can be found with strigolactones in general. For example, all of the compounds were known, before the present application was filed, to be able to trigger the germination of weed plants.

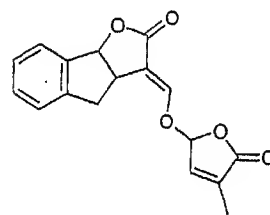
The claimed group of compounds also maintains a particular structure and configuration of rings denoted B, C and D, as shown below in the general strigol molecule.



One of ordinary skill in the art could reasonably expect that similar compounds having such a common structure,



analogous to GR7



and to GR24

would have similar properties. That is to say, any compound having the structure of the chain of rings denoted B, C and D of strigolactones (e.g., demethylsorgolactone) or their configuration (e.g., Nijmegen-1, another synthetic strigolactone analog), would be capable of stimulating the development and/or growth of AM fungi. (See, specification, page 7, line 27 to page 8, line 16).

Thus, in contrast to the position taken in the Office Action, the claims do not include a broad group of many unrelated stimulating agents.

### Working Examples

The Office Action takes the position that the specification provides only limited working examples and does not exemplify all nine of the strigolactone compounds.

The specification includes the analysis of synthetic strigolactones GR7 and GR24 on *Glomus intraradices* and *Gigaspora rosea*, two familiar types of arbuscular mycorrhiza fungi. The AM fungus *G. intraradices* is a commonly used soil inoculant in agriculture and horticulture and is one of the best mycorrhizal varieties of fungi available for use in mycoforestry. The *G. intraradices* is perhaps the most commonly used variety of arbuscular mycorrhizal fungi in scientific studies of the effects of arbuscular mycorrhizal fungi on plant and soil improvement. (See, Wikipedia, [http://en.wikipedia.org/wiki/Glomus\\_intraradices](http://en.wikipedia.org/wiki/Glomus_intraradices)).

The examples in the specification demonstrated that GR7 and GR24 had effects on the both the activation of cell respiration and the stimulation of branching of hyphae of AM fungi. The examples also showed that GR7 had effects on the mycorrhization of *Medicago trunculata* by AM fungus. *M. trunculata* is a small legume that is also a model organism for legume biology.

Thus, the specification exemplifies two representative members of the common strigolactone family (i.e., GR7 and GR24) in experiments with established model organisms. One of skill in

the art would accept that these experiments would very likely represent the effects common to all nine of the claimed strigolactone compounds.

Unpredictability

The Office Action appears to take the position that because the natural strigolactones strigol, sorgolactone and alethrol are known to be difficult to synthesize economically that this makes them unpredictable. Applicants respectfully do not understand the relevance of this line of reasoning. The difficult synthesis and purification of these compounds is known and recognized in the art but this does not take away from the ability of these strigolactones to serve as stimulating agents for stimulating the development and/or growth of AM fungi.

First, all of the strigolactone compounds of claim 23 are known to trigger the germination of weed plants as evidenced by the following references of record:

- GR24: MANGNUS et al., (1992), J. Agric. Food Chem., 40(6), 697-700;
- GR7: JOHNSON et al., (1976), Weed Res., 16,223-227;
- Nijmegen-1: NEFKENS et al., (1997), J. Agric. Food Chem., 45(6), 2273-2277;
- Demethylsorgolactone: THURING et al., (1997), J. Agric. Food Chem., 45(6), 2278-2283;
- Strigol: COOK et al., (1996), Science, 1994, 1189-1190;
- Sorgolactone: HAUCK et al., (1992), J. Plant Physiol., 139, 474;

- Alectrol: MÜLLER et al., (1992), J. Plant Physiol., 11, 77; WIGCHERT et al., (1999), J. Agric. Food Chem., 47, 1705-1710;

- Orobanchol: YOKOTA et al., (1998), Phytochemistry, 49(7), 1967-1973.

One of ordinary skill in the art would readily recognize that these compounds all behave similarly in weed plants and that these compounds may be useful to obtain the claimed effects with AM fungi, i.e. stimulation of development and growth.

Second, despite whatever difficulty one might have in "economically" obtaining the strigolactones, one of ordinary skill would have been able to do so. The compounds can be synthesized and purified as evidenced by the following references of record:

- NEFKENS et al., (1997) J. Agric. Food Chem., 45(6), 2273-2277, teaches that (+)-strigol can be efficiently isolated from the root exudate of the false host cotton (COOK et al., (1996), Science, 1954, 1189-1190);

- HAUCK et al., J. Plant Physiol., (1992) 139, 474 describes the chemical synthesis of Sorgolactone (the strigolactone of sorghum). The present application also discloses that 5µg of sorgolactone can be purified from sorghum plants;

- MÜLLER et al., (1992) J. Plant Physiol, 11, 77 describes alectrol.

All of these references were of record at the time the present application was filed. Furthermore, sorgolactone and Nijmegen-1 were commercially available from CHIRALIX (Netherlands) at the time of filing. Applicants enclose a copy of an invoice, dated February 11, 2005, as evidence of the commercial availability of a mixture of sorgolactone diastereomers.

Thus, the position taken in the Office Action, concerning the availability of strigolactones for one of ordinary skill to perform the necessary experiments, is untenable.

Third, in regard to predictability, the Office Action contends that the "complexity" of strigolactones "results in instability in soil" and that "strigol is unstable in alkaline soils." Again, this line of reasoning has little relevance to the presently claimed methods of treating AM fungi.

NEFKENS et al. states that "an attractive control strategy for the eradication of infested fields is the concept of suicidal germination, i.e., introduction of a germination stimulating agent into the soil prior to sowing to induce germination of the parasitic seeds in the absence of the host plant." (See, Introduction, page 2273, first paragraph). NEFKENS et al. concludes, however, that strigol is not economically adapted to be introduced into the soil of infested fields and that strigolactones "are not suitable for weed control purpose in field." (See, page 2273, second paragraph).

In distinction, however, the present claims are directed to a method of treating arbuscular mycorrhiza (AM) fungi. The examined claims do not feature spreading strigolactones in soil. As detailed in the specification, the treated AM fungi can be spread on soil, "the spreading of AM fungi on cultivation soils would optimize their content of these microorganisms that are beneficial to the plant to be cultivated." (See, specification, page 2, lines 26-30).

Contrary to the reasoning taken by the Office, it is not a purpose of the present invention to introduce strigol or strigol analogs directly into the soil of fields. The invention focuses on preparing large quantities of AM fungi, preferably in the form of spores, which can then be spread in fields to elicit a beneficial effect on the growth of cultivated plants. One of ordinary skill in the art may have been dissuaded to use strigol as a substance to be spread directly in the fields (i.e., suicide germination of parasitic weeds) but one was enabled to use reduced quantities of strigol and strigol analogues to treat AM fungi and then spread the treated AM fungi in the soil.

#### Quantity of Experimentation

The Office Action contends that one of skill would have to find out if seven of the nine compounds (Nijmegen 1, Demethylsorgolactone, Strigol, Sorgolactone, Alectrol, and Orobanchol) would stimulate the development and/or growth of AM



fungi and that this would require additional experimentation. In contrast to the position taken by the Office, however, this would not require undue experimentation.

In support of this position, Applicants submit herewith two additional documents to show that this type of experimentation is essentially routine.

- AKIYAMA et al., (2010), Plant Cell Physiol., 51 (7), 1104-1117;
- BESSERER et al. (2006), PLOS Biology, 4(7), 1239-1247.

AKIYAMA et al. determined that strigolactones induce hyphal branching in AM fungi at very low concentrations "suggesting a highly sensitive perception system for strigolactones present in AM fungi." (See, Abstract). AKIYAMA tested a series of natural and synthetically modified strigolactones as well as non-strigolactone-type germination stimulants for hyphal branching-inducing activity in *Gigaspora margarita*, and determined the minimum effective concentration of GR24, GR7, strigol, orobanchol and sorgolactone on the hyphal branching-inducing activity. In other words, AKIYAMA was able to easily do what the Office Action contends is undue experimentation.

BESSERER et al. studied the quantitative hyphal branching response of AM fungus stimulated by strigolactones such as GR24, GR7 and sorgolactone. BESSERER determined that the strigolactones strongly and rapidly stimulated cell proliferation

of the AM fungus *Gigaspora rosea* at concentrations as low as  $10^{-13}$  (See, Table 1). The strigolactones also stimulated spore germination of two other phylogenetically distant AM fungi, *Glomulus intraradices* and *Gl. claroideum*. From these studies, BESSERER was able to conclude that "strigolactones are important rhizospheric plant signals involved in stimulating both the pre-symbiotic growth of AM fungi and the germination of parasitic plants." (See, Abstract). Again, BESSERER was able to determine by successful experimentation the effects of the strigolactone family of compounds on AM fungi along with their necessary dosage levels.

Thus, contrary to the position taken in the Official Action, one of ordinary skill could have determined the ability of all strigolactones recited in the present claims to stimulate the growth and development of AM fungi, without undue experimentation.

For all of these reasons, the specification fully enables the full scope of claim 23 and claims 24-30 dependent thereon.

#### **NEW CLAIM 45**

New claim 45 is directed to the method of claim 23, wherein the stimulating agent is defined to be GR24 or GR7. At page 4, the Office Action acknowledges that claim 45 is directed to allowable subject matter.

**CONCLUSION**

Consideration of the above remarks is earnestly solicited. Applicants respectfully request that a timely Notice of Allowance be issued in this case.

Should there be any matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number listed below.

The fee of \$52 for the one extra dependent claim added herewith, is being paid concurrently online by credit card.

The Commissioner is hereby authorized in this, concurrent, and future submissions, to charge any deficiency or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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**APPENDIX:**

The Appendix includes the following item(s):

CHIRALIX invoice;

AKIYAMA et al., (2010), Plant Cell Physiol., 51 (7), 1104-1117;

BESSERER et al. (2006), PLOS Biology, 4(7), 1239-1247.